NOVEL TRIPEPTIDE, ITS PRODUCTION AND HYPOTENSOR CONTAINING THE SAME AS AN ACTIVE INGREDIENT

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Abstract of **JP7188282**

PURPOSE:To obtain a novel tripeptide which is useful as a hypotensor with high safety, low toxicity and no anaphylaxy shock.
CONSTITUTION:Twenty three kinds of tripeptide having the L-amino acid sequences are represented by the formulas. The tripeptides are obtained by treating sardin muscles with a protease, filtering the product, and fractionating the components passing through the semipermeable membrane by means of a strong acid cation exchange resin, gel filtration, ion-exchange gel filtration, reverse-phase high-performance liquid chromatography in order to collect the fractions containing the components having the activity of inhibiting the enzymes transforming angiotensin.

Leu-Ala-Phe, Val-Ala-Tyr, Met-Val-Val,
Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,
Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

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(54)【発明の名称】　新規なトリペプチド、その製法およびそれを有効成分と　する血圧降下剤

(57)【要約】

【目的】　イワシ筋肉並びに大豆タンパク質分解酵素の分解液から新規な血圧降下作用を有するペプチドを提供する。

【構成】　イワシ筋肉をタンパク質分解酵素等で処理し、アンジオテンシン変換酵素阻害活性を有する新規な２３種のトリペプチドを単離した。又、大豆をタンパク質分解酵素等で処理し、アンジオテンシン変換酵素阻害活性を有する２種のトリペプチドを単離した。

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【特許請求の範囲】　　＊　＊【請求項1】

次式；　Leu-Ala-Phe,　Val-Ala-Tyr,　Met-Val-Val,
Val-Val-Leu,　Ala-Ala-Phe,　Leu-Ala-His,
Leu-Glu-Leu,　Ala-Tyr-Val,　Ala-Val-Met,
Ala-Val-Lys,　Glu-Val-Tyr,　Gly-Val-Leu,
Tyr-Asp-Ala,　Leu-Trp-Trp,　Leu-Ala-Ala,
Glu-Ala-Val,　Phe-Ile-Leu,　Ala-Leu-Ala,
Thr-Gly-Pro,　Met-Gly-Ile,　Leu-Ala-Val,
Leu-Val-Val,　Asn-Gln-Phe,

で示されるL体のアミノ酸の配列によるペプチド構造を有する新規な２３種類のトリペプチド。

【請求項2】　イワシ筋肉をタンパク質分解酵素で処理して得られた生成物を沪過し、その濾液成分中の半透膜を通過した成分を順次、強酸性陽イオン交換樹脂、ゲル沪過、イオン交換性ゲル沪過、逆相高速液体クロマトグラフィーによって分画し、その処理毎に得られた分画か※らアンジオテンシン変換酵素阻害活性を有する成分を含有する分画を得ることを特徴とする請求項1の新規な２３種のトリペプチドの製法。

【請求項3】　請求項1の新規な２３種のトリペプチドから選ばれた１種以上のトリペプチドを有効成分とする血圧降下剤。

【請求項4】

次式；　Ala-Ile-Met,　Tyr-Ala-Val,　Gly-Gly-Phe,
Gln-Gly-Phe,　Leu-Glu-Leu,　Tyr-Ala-Phe,
Gly-Tyr-Ile,　Tyr-Glu-Phe,　Ala-Asp-Tyr,
Glu-Gly-Gln,　Gln-Phe-Ala,　Phe-Met-Gly,
Gly-Phe-Gly,　Ile-Gly-Ser,　Trp-Trp-Leu,
Ala-Ala-Leu,　Leu-Ileu-Phe,　Ala-Leu-Ala,
Pro-Gly-Thr,　Phe-Leu-Met,　Trp-Ala-Pro,
Tyr-Ile-Ala,　Phe-Ser-Pro,　Phe-Phe-Tyr,
Phe-Val-Ala,　Gly-Phe-Ile,　Ala-Ala-Val,

で示されるL体のアミノ酸の配列によるペプチド構造を有する新規な２７種のトリペプチド。

【請求項5】　大豆をタンパク質分解酵素で処理して得られた生成物を沪過し、その沪過成分中の半透膜を通過した成分を順次、強酸性陽イオン交換樹脂、ゲル濾過、イオン交換性ゲル沪過、逆相高速液体クロマトグラフィーよって分画し、その処理毎に得られた分画からアンジオテンシン変換酵素阻害活性を有する成分を含有する分画を得ることを特徴とする請求項1の新規な２７種のトリペプチドの製法。

【請求項6】　請求項4の新規な２７種のトリペプチドから選ばれた１種以上のトリペプチドを有効成分とする血圧降下剤。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、新規なトリペプチドを有効成分とする血圧降下剤およびその新規なトリペプチドの製法に関するものである。

【0002】

★【従来の技術】高血圧は、病因的に血圧上昇の原因が明らかなもの（病候性高血圧）と不明なもの（本態性高血圧）とに大別されている。病候性高血圧は原因となる疾患を治癒させることで高血圧を治癒させることができるが、本態性高血圧では原因に対する直接的な治療法は困難である。従来、レニン－アンジオテンシン系（以下、Ｒ・Ａ系と略記する。）は、本態性高血圧の重要な要因の一つであると考えられており、ここ１０年来、Ｒ・Ａ系で中心的な役割を果たしているアンジオテンシン変換酵素（以下、ＡＣＥと略記する。）の活性を阻害することによってＲ．Ａ系を調節して本態性高血圧を調節する試みが行われてきた。そのようなＡＣＥ活性阻害を有する物質としては、合成化合物の場合にはＬ－プロリン誘導体［Ｍ．Ａ．Ｏｎｄｅｔｔｉ，Ｂ．Ｒｕｂｉｎ ｅｔ ａｌ；Ｓｃｉｅｎｃｅ，１９６，４４１（１９７７）］やそれをベースにした化合物が知られており、天然物由来の物質の場合には蛇毒由来のブラディキニン増強因子（Ｃ末端がＰｒｏ）［Ｓ．Ｈ．Ｆｅｒｒｅｉａ，Ｄ．Ｃ．Ｂａｒｔｅｌｔ ｅｔ ａｌ；Ｂｉｏｃｈｅｍ

istry, 9, 3583 (1970)]、ゼラチンのコラゲナーゼ消化物由来の6種類のペプチド（C末端がAla−Hyp）[G. Oshima, H. Shimabukuro et al; Biochim. BioPhs, Acta, 566, 128 (1979)]、牛カゼインのトリプシン消化物由来のペプチド（C末端がGly−Lys）[S. Maruyama, H. Suzuki; Agric. Biol. Chem, 46, 1393 (1982)]などが知られている。食品の場合には鈴木らが大豆、茶類、貝類、果実類などでACE活性阻害を認めている［鈴木健夫、石川宣子ら；農化，57，1143（1983）］。しかし、これら天然物由来の物質はいずれも静脈内投与で効果が確認されている＊

＊のみで、経口投与による薬理効果は不明であり、発明されてから長期間経過しているが、未だ医薬品としての開発が進んでいるとの報告はない。

【0003】

【発明が解決しようとする課題】本発明の目的は、新規なトリペプチド、その製法およびそれを有効成分とする血圧降下剤を提供することである。

【0004】

【課題を解決するための手段】本発明は、前記の課題を解決するために鋭意研究した結果、イワシ筋肉ならびに大豆のタンパク質分解酵素の分解液から得られた本発明の新規なペプチドが、血圧降下作用を有することを見出し、本発明を完成するに至った。即ち、本発明は、

(1)次式；　Leu-Ala-Phe,　Val-Ala-Tyr,　Met-Val-Val,
Val-Val-Leu,　Ala-Ala-Phe,　Leu-Ala-His,
Leu-Glu-Leu,　Ala-Tyr-Val,　Ala-Val-Met,
Ala-Val-Lys,　Glu-Val-Tyr,　Gly-Val-Leu,
Tyr-Asp-Ala,　Leu-Trp-Trp,　Leu-Ala-Ala,
Glu-Ala-Val,　Phe-Ile-Leu,　Ala-Leu-Ala,
Thr-Gly-Pro,　Met-Gly-Ile,　Leu-Ala-Val,
Leu-Val-Val,　Asn-Gln-Phe,

で示されるL体のアミノ酸配列を有する新規な２３種のトリペプチド。

【0005】（２）イワシ筋肉をタンパク質分解酵素で処理して得られた生成物を濾過し、その沪過成分中の半透膜を通過した成分を順次、強酸性陽イオン交換樹脂、ゲル沪過、イオン交換性ゲル沪過、逆相高速液体クロマ※トグラフィーによって分画し、その処理毎に得られた分画からアンジオテンシン変換酵素阻害活性を有する成分を含有する分画を得ることを特徴とする前記の新規な２３種のトリペプチドの製法。

（３）前記の新規なトリペプチドを有効成分とする血圧降下剤。

(4)次式；　Ala-Ile-Met,　Tyr-Ala-Val,　Gly-Gly-Phe,
Gln-Gly-Phe,　Leu-Glu-Leu,　Tyr-Ala-Phe,
Gly-Tyr-Ile,　Tyr-Glu-Phe,　Ala-Asp-Tyr,
Glu-Gly-Gln,　Gln-Phe-Ala,　Phe-Met-Gly,
Gly-Phe-Gly,　Ile-Gly-Ser,　Trp-Trp-Leu,
Ala-Ala-Leu,　Leu-Ileu-Phe,　Ala-Leu-Ala,
Pro-Gly-Thr,　Phe-Leu-Met,　Trp-Ala-Pro,
Tyr-Ile-Ala,　Phe-Ser-Pro,　Phe-Phe-Tyr,
Phe-Val-Ala,　Gly-Phe-Ile,　Ala-Ala-Val,

で示されるL体のアミノ酸配列を有する新規な２７種のトリペプチド。

【0006】（５）大豆をタンパク質分解酵素で処理して得られた生成物を濾過し、その沪過成分中の半透膜を通過した成分を順次、強酸性陽イオン交換樹脂、ゲル沪過、イオン交換性ゲル濾過、逆相高速液体クロマトグラフィーによって分画し、その処理毎に得られた分画から★

次式；　Leu-Ala-Phe,　Val-Ala-Tyr,　Met-Val-Val,

★アンジオテンシン変換酵素阻害活性を有する成分を含有する分画を得ることを特徴とする前記の新規な２７種のトリペプチドの製法。

（６）前記の新規なトリペプチドを有効成分とする血圧降下剤に関するものである。以下、本発明を詳細に説明する。本発明の新規なトリペプチドは、

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Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,
Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

（以上２３種、トリペプチドの式中の各記号はペプチド化学におけるアミノ酸配列の各アミノ酸単位を示す。）＊

Ala-Ile-Met, Tyr-Ala-Val, Gly-Gly-Phe
Gln-Gly-Phe, Leu-Glu-Leu, Tyr-Ala-Phe
Gly-Tyr-Ile, Tyr-Glu-Phe, Ala-Asp-Tyr
Glu-Gly-Gln, Gln-Phe-Ala, Phe-Met-Gly
Gly-Phe-Gly, Ile-Gly-Ser, Trp-Trp-Leu
Ala-Ala-Leu, Leu-Ileu-Phe, Ala-Leu-Ala
Pro-Gly-Thr, Phe-Leu-Met, Trp-Ala-Pro
Tyr-Ile-Ala, Phe-Ser-Pro, Phe-Phe-Tyr
Phe-Val-Ala, Gly-Phe-Ile, Ala-Ala-Val

（以上２７種、トリペプチドの式中の各記号はペプチド化学におけるアミノ酸配列の各アミノ酸単位を示す。）で示されるＬ体のアミノ酸配列を有する新規なトリペプチドであり、この常温における性状は白色粉末である。

【０００８】前記の新規なトリペプチドの製法としては、そのトリペプチドを化学的に合成する方法またはイワシ筋肉並びに大豆のタンパク質分解酵素の分解液から分離、精製する方法を挙げることができる。本発明の新規なトリペプチドを化学的に合成する場合には、液相法または固相法などの通常の合成方法によって行うことができるが、好ましくは、固相法によってポリマー性の固相支持体へ前記トリペプチドのＣ末端側（カルボキシル末端側）からそのアミノ酸残基に対応したＬ体のアミノ酸を順次ペプチド結合によって結合して行くのが良い。そして、そのようにして得られた合成トリペプチドは、トリフルオロメタンスルホン酸、フッ化水素などを用いてポリマー性の固相支持体から切断した後、アミノ酸側鎖の保護基を除去し、逆相系のカラムを用いた高速液体クロマトグラフィー（以下、ＨＰＬＣと略す。）などを用いた通常の方法で精製することができる。

【０００９】本発明の新規なトリペプチドを、イワシ筋肉並びに大豆のタンパク質分解酵素の分解液から分離精製することができるが、その場合には１９９１年度日本農芸化学会大会（京都）講演要旨集Ｐ１８３　３ＡＰ１３の方法に準拠し、例えば以下のようにして行うことができる。上記の新規なトリペプチドを含有しているイワシ筋肉部分並びに大豆を取り出して、ホモゲナイザーを用いて適当な溶媒（例えば、水、トリス－塩酸緩衝液、※リン酸緩衝液などの中性の緩衝液など）中で十分にホモジネートした後、加水分解する。加水分解は常法に従って行う。例えば、ペプシン等タンパク質分解酵素で加水分解する場合は、イワシ筋肉ホモジネート並びに大豆ホモジネートを必要とあれば更に加水分解した後、酵素の至適温度まで加温し、ｐＨを至適値に調整し、酵素を加えてインキュベートする。次いで必要に応じ中和した後、酵素を失活させて加水分解液を得る。その加水分解物を沪紙およびセライトなどを用いて沪過することによって不溶性成分を除去し、その得られた濾液をセロファンなどの半透膜を用いて適当な溶媒（例えば、水、トリス－塩酸緩衝液リン酸緩衝液などの中性の緩衝液など）中で十分に透析し、その濾液中の成分で半透膜を通過した成分を含む溶液を強酸性陽イオン交換樹脂（例えば、ダウケミカル社製のＤｏｗｅｘ　５０Ｗなど）にかけ、その吸着溶出分画からアンジオテンシン変換酵素（以下、ＡＣＥと略す。）阻害活性を有する成分を含有する分画を得、その得られたＡＣＥ阻害活性分画をゲル濾過（例えば、ファルマシア製の　Ｓｅｐｈａｄｅｘ　Ｇ－２５など）によって分画し、その得られたＡＣＥ阻害活性分画を陽イオン交換ゲル沪過（例えば、ファルマシア製のＳＰ－Ｓｅｐｈａｄｅｘ　Ｃ－２５など）によって分画し、その得られたＡＣＥ阻害活性分画をさらにＨＰＬＣ（逆相高速液体クロマトグラフィー）によって分画することによって行うことができる。

【００１０】本発明の新規なトリペプチドの製法において用いる魚筋肉並びにマメ科植物としては、本発明の目的を達成できる限りいかなる魚筋肉並びにマメ科植物を

用いても良いが、好ましくはイワシ並びに大豆を用いるのが良い。以上のようにして得られた本発明の新規なトリペプチドは、静脈内へ繰り返し投与しても抗体産生を惹起せず、また、アナフィラキシーショックを起こさせない。また、本発明の新規なトリペプチドはL－アミノ酸のみの配列構造からなり、その分子サイズからみて、投与後、生体内のプロテアーゼにより分解されることなく、すみやかに腸管吸収され、その血圧降下作用を発揮するため毒性は極めて低く、安全性は極めて高い（LD_{50}）5000kg／kg；ラット経口投与）。本発明に係る新規なトリペプチドは、通常用いられる賦形剤等の添加物を用いて注射剤、錠剤、カプセル剤、顆粒剤、散剤等に調整することができる。投与方法としては、通常は、ACEを有している哺乳類（例えば、ヒト、イヌ、ラット等）に注射すること、あるいは経口投与することがあげられる。投与量は、例えば、動物体重1kg当りこのトリペプチドを0．01～10mgの量である。投与回数は、通常1日1～4回程度であるが、投与経路によって、適宜、調整することができる。本発明に係る新規なトリペプチドは優れたアンジオテンシン変換酵素阻害作用を有し、血圧降下作用、ブラジキニン不活化抑制作用を示す。したがって、本態性高血圧、腎性高血圧、副腎性高血圧等の高血圧症の予防、治療剤、これらの疾患の診断剤や各種の病態において用いられる血圧降下剤として有用であり、更にうっ血性心不全に対する臓器循環の正常化と長期予後の改善（延命効果）作用を有し、心不全の治療剤として有用である。

【実施例】以下に実施例として、製造例および試験例を記載し、本発明を更に詳細に説明する。

【0011】製造例1

［新規なトリペプチドのイワシ筋肉からの製造］イワシ筋肉500gに脱イオン水1Lを加え、ホモジナイズした後、1N塩酸にてpHを2．0に調整し　ペプシン（メルク社製、酵素番号EC3．4．23．1）10gを添加し、37℃20時間撹拌しながら加水分解を行った。分解反応液を直ちに限外戸過膜（アミコン社製、Y＊＊M10型、φ76mm）に通過させ、通過液をDowex　50W×4［H^+］カラム（φ4．5x15cm）に加えた。そのカラムを脱イオン水で十分洗浄した後、2N水酸化アンモニウム液2Lを用いて溶出した。減圧濃縮によりアンモニアを除去し、濃縮液40mlを得た。この濃縮液4mlを予め脱イオン水で緩衝化したSephadex　G－25カラム（φ2．5x150cm）に負荷し、流速30ml／hr，各分画量8．6mlでゲル戸過を行った。ゲル濾過を繰り返して大量分取したACE阻害活性の高い画分を集め凍結乾燥してペプチド粉末とした。このペプチド3gを20mlの脱イオン水に溶解後、予め、脱イオン水で緩衝化したSP－Sephadex　C－25（H^+）カラム（φ1．5x47．2cm）に負荷し、脱イオン水1Lから3%塩化ナトリウム液1Lの濃度勾配法を行い、流速3ml／hr，各分画量10．0mlでクロマトグラフィーを行った。その結果は図1に示すとおりである。上記クロマトグラフ中、分画番号22～28のACE阻害活性分画を集めて凍結乾燥して精製トリペプチド粉末を得た。この精製トリペプチド粉末20mgを60μlの脱イオン水に溶解した後、HPLCを行った。カラムとしては野村化学（株）製DevelosilODS－5（4．5mmIDx25cmL）を使用し、移動相としては0．05%トリフルオロ酢酸（以下、TFAと略記する。）から25%アセトニトリル／0．05%TFAの濃度勾配法を行い、流速1．0ml／min，検出波長220nmでクロマトグラフィーを行い、ACE阻害作用を有するトリペプチドを得た。その結果は図2に示すとおりであり、23種のトリペプチドの溶出時間は表1のとおりである。

【0012】このようにして得られたACE阻害作用を有するトリペプチドのアミノ酸配列は、アプライドバイオシステム社製のプロテインシークエンサー447A型を用いて決定された。その結果、23種のトリペプチドはそれぞれ、

次式；　Leu-Ala-Phe，　Val-Ala-Tyr，　Met-Val-Val，
Val-Val-Leu，　Ala-Ala-Phe，　Leu-Ala-His，
Leu-Glu-Leu，　Ala-Tyr-Val，　Ala-Val-Met，
Ala-Val-Lys，　Glu-Val-Tyr，　Gly-Val-Leu，
Tyr-Asp-Ala，　Leu-Trp-Trp，　Leu-Ala-Ala，
Glu-Ala-Val，　Phe-Ile-Leu，　Ala-Leu-Ala，
Thr-Gly-Pro，　Met-Gly-Ile，　Leu-Ala-Val，
Leu-Val-Val，　Asn-Gln-Phe，

で示されるL体のアミノ酸残基からなる配列を有するトリペプチドであることが確認された。新規23種のトリペプチドをマススペクトルにより分析した結果、アミノ酸配列およびアミノ酸組成が前記式で示したアミノ酸配※列構造を有するトリペプチドであることが確認された。このマススペクトルの結果は表1に示すとおりである。

【0013】製造例2

［新規なトリペプチドの大豆からの製造］大豆200g

に脱イオン水1Lを加え、ホモジナイズした後、1N塩酸にてpHを2．0に調整し、ペプシン（メルク社製、酵素番号EC3．4．23．1）10gを添加し、37℃20時間撹拌しながら加水分解を行った。分解反応液を直ちに限外濾過膜（アミコン社製、YM10型、φ76mm）に通過させ、通過液をDowex　50W×4［H^+］カラム（φ4．5x15cm）に加えた。そのカラムを脱イオン水で十分洗浄した後、2N水酸化アンモニウム液2Lを用いて溶出した。減圧濃縮によりアンモニアを除去し、濃縮液40mlを得た。この濃縮液4mlを予め脱イオン水で衝衝化したSephadex G－25（φ2．5x150cm）に負荷し、流速30ml／hr，各分画量8．6mlでゲル沪過を行った。ゲル沪過を繰り返して大量分取したACE阻害活性の高い分画を集め凍結乾燥してペプチド粉末とした。このペプチド3gを20mlの脱イオン水に溶解後、予め、脱イオン水で緩衝化したSP－Sephadex　C－25［H^+］カラム（φ1．5x47．2cm）に負荷し、脱イオン水1Lから3%塩化ナトリウム液1Lの濃度勾配法を行い、流速3ml／hr，各分画10．0m＊1でクロマトグラフィーを行った。その結果は図3に示すとおりである。

【0014】上記クロマトグラフ中、分画番号32～38のACE阻害活性分画を集めて凍結乾燥して精製トリペプチド粉末を得た。この精製トリペプチド粉末20mgを60μlの脱イオン水に溶解した後、HPLCを行った。カラムとしては野村化学（株）製Develosil ODS－5（4．5mmIDx25cmL）を使用し、移動相としては0．05%トリフルオロ酢酸（以下TFAと略記する。）から25%アセトニトリル／0．05%TFAの濃度勾配法を行い、流速1．0ml／min，検出波長220nmでクロマトグライーを行い、ACE阻害作用を有するリペプチドを得た。その結果は図4に示すとおりであり、27種のトリペプチドの溶出時間は表2のとおりである。

【0015】このようにして得られたACE阻害作用を有するトリペプチドのアミノ酸配列は、アプライドバイオシステム社製のプロテインシークエンサー477A型を用いて決定された。その結果、27種のトリペプチドはそれぞれ、

次式；　Ala-Ile-Met，　Tyr-Ala-Val，　Gly-Gly-Phe，
Gln-Gly-Phe，　Leu-Glu-Leu，　Tyr-Ala-Phe，
Gly-Tyr-Ile，　Tyr-Glu-Phe，　Ala-Asp-Tyr，
Glu-Gly-Gln，　Gln-Phe-Ala，　Phe-Met-Gly，
Gly-Phe-Gly，　Ile-Gly-Ser，　Trp-Trp-Leu，
Ala-Ala-Leu，　Leu-Ileu-Phe，　Ala-Leu-Ala，
Pro-Gly-Thr，　Phe-Leu-Met，　Trp-Ala-Pro，
Tyr-Ile-Ala，　Phe-Ser-Pro，　Phe-Phe-Tyr，
Phe-Val-Ala，　Gly-Phe-Ile，　Ala-Ala-Val，

で示されるL体のアミノ酸残基からなる配列を有するトリペプチドであることが確認された。新規27種のトリペプチドをマススペクトルにより分析した結果、アミノ酸配列およびアミノ酸組成が前記式で示したアミノ酸配列構造を有するトリペプチドであることが確認された。このマススペクトルの結果は表2に示すとおりである。精製して得られた本発明に係るイワシ筋肉由来トリペプチド23種より成る分画、並びに大豆由来トリペプチド27種より成る分画は、以下に示す試験によって薬理効果が確認された。

【0016】試験例1

［ACE阻害活性測定法］ACE（シグマ社製、酵素番号EC3．4．15．1）2．5mU，合成基質Hippuryl－L－his－tidyl－L－leucine（ペプチド研究所製）12．5mMを用いLiebermanの測定法を改良した山本等の方法（日胸疾会誌，18，297－302（1989））に準じて測定した。すなわち、生成した馬尿酸を酢酸エチルにて抽出し、225nmの吸光度で測定した。被検液での吸光度※をEs，被検液の代わりに緩衝液を加えた時の値をEc，予め反応停止液を加えて反応させた時の値をEbとして次式から阻害率を求めた。

阻害率（%）＝（Ec－Es）／（Ec－Eb）×100

ACE阻害剤の阻害活性IC_{50}値は、ACEの酵素活性を50%（阻害率）阻害するために必要な試料の濃度（M）で示した。本発明に係るイワシ筋肉由来新規23種のトリペプチドの牛肺血清ACEに対する阻害活性（IC_{50}）は表1に示すとおりである。また、本発明に係る大豆由来新規27種のトリペプチドの牛肺血清ACEに対する阻害活性（IC_{50}）は表2に示すとおりである。

【0017】試験例2

［新規なトリペプチドのラットへ投与時の降圧の効果］

Ⅰ．実験材料

前記製造例1，2で得られた精製トリペプチド粉末。すなわちイワシ筋肉由来トリペプチド23種より成る分画（SP－1分画）並びに大豆由来トリペプチド27種よ

り成る分画（ＳＰ－１分画）を用いた。

ＩＩ．実験方法

実験動物は日本チャールズ・リバー（株）より１５週令雄性高血圧自然発症ラット（ＳＨＲ）を購入し、１週間の予備飼育後、収縮期血圧が１６０ｍｍＨｇ以上（体重２８０～３３０ｇ）の動物６匹１群として用いた。ラットは、室温２３±２５℃、湿度５５±１０％および１２時間明暗（午前６時～６時点灯）に調整された飼育室でステンレスワイヤー製ラット用個別ケージに１匹ずつ収容し飼育した。飼料はオリエンタル酵母工場（株）製ＭＦ粉末飼料を、飲水は自家揚水（水道水質基準適合）をそれぞれ自由に摂取させた。ラットは４群（１群６匹）に分け、第１群には対照として蒸留水を体重１００ｇあたり０．５ｍｌの割合で強制経口投与した、第２群にはトリペプチドの粉末１．０ｇ／ｋｇの用量を蒸留水で調製し、体重１００ｇあたり０．５ｍｌの割合で強制経口投与し、第３群にはトリペプチドの粉末２．０ｇ／ｋｇ、第４群にはトリペプチドの粉末４．０ｇ／ｋｇの用量を、第２群と同様に強制経口投与した。

【００１８】血圧は非観血的尾動脈血圧測定装置（（株）理研開発製、ＰＳ－１００）を用いｔａｉｌ－ｃｕｆｆ法により、投与前、投与後３０分、１時間、２時間、４時間および６時間の血圧および心拍数を測定した。血圧は連続３回測定し、その最高値と最低値の差が１０ｍｍＨｇ以内の場合、その３回の平均血圧値を求めた。差が１１ｍｍＨｇ以上の場合はさらに２回測定し、最高値および最低値を除き３回の平均血圧値を求めた。また、平均心拍数は平均血圧値を算出したときの測定値を用いて求めた。ＳＨＲを用いて、イワシ筋肉由来トリペプチド分画（ＳＰ－１分画）３００，６００および１，２００ｍｇ／ｋｇを単回経口投与した時の、血圧値および心拍数への作用についての結果は、表３，図５に示すとおりである。またＳＨＲを用いて大豆由来トリペプチド分画（ＳＰ－１分画）３００，６００および１，２００ｍｇ／ｋｇを単回経口投与した時の、血圧値および心拍数への作用についての結果は、表４、図６に示すとおりである。以上の試験の結果、本発明に係るイワシ筋肉由来トリペプチド２３種より成る分画並びに大豆由来トリペプチド２７種より成る分画は、ＡＣＥ阻害活性を有し、ｉｎ　ｖｉｖｏにおいても有意な血圧降下作用を示すことが確認された。したがって、本発明に係るイワシ筋肉由来トリペプチド２３種並びに大豆由来トリペプチド２７種は高血圧症の治療または予防薬として有用である。なお、本発明に係るイワシ筋肉由来トリペプチド２３種並びに大豆由来トリペプチド２７種は、構造的にそのアミノ酸配列を部分構造とするペプチドにおいて、構造中に採用することもできる。

【００１９】

【表１】

13　　14

HPLCにおける溶出時間（分）	アミノ酸配列	分子量（MH⁺）	阻害活性 IC_{50}値（$\times 10^{-5}$M）
20.0	Leu-Ala-Phe	350	5.3
21.8	Val-Ala-Tyr	352	4.2
30.7	Met-Val-Val	348	3.1
30.9	Val-Val-Leu	330	2.8
31.3	Ala-Ala-Phe	308	9.2
31.6	Leu-Ala-His	340	8.3
31.8	Leu-Glu-Leu	374	1.3
31.9	Ala-Tyr-Val	352	1.7
32.1	Ala-Val-Met	320	0.8
32.6	Ala-Val-Lys	317	1.6
32.7	Glu-Val-Tyr	410	8.1
32.8	Gly-Val-Leu	288	8.8
33.8	Tyr-Asp-Ala	368	4.7
44.8	Leu-Trp-Trp	504	5.6
44.9	Leu-Ala-Ala	274	6.1
55.2	Glu-Ala-Val	318	6.8
55.9	Phe-Ile-Leu	392	1.9
60.0	Ala-Leu-Ala	274	3.8
60.1	Thr-Gly-Pro	274	5.3
60.2	Met-Gly-Ile	320	5.6
72.0	Leu-Ala-Val	302	4.8
72.5	Leu-Val-Val	330	6.5
88.4	Asn-Gln-Phe	408	4.9

イワシ筋肉由来トリペプチドのＨＰＬＣにおける溶出時間、アミノ酸配列、分子量および阻害活性。　＊【００２０】　＊【表２】

15　　16

HPLCにおける溶出時間（分）	アミノ酸配列	分子量（MH⁺）	阻害活性 IC_{50}値（$\times 10^{-5}$M）
19.1	Ala-Ile-Met	334	0.3
21.8	Tyr-Ala-Val	352	4.6
26.6	Gly-Gly-Phe	280	2.1
31.6	Gln-Gly-Phe	351	6.7
31.8	Leu-Glu-Leu	374	8.1
31.9	Tyr-Ala-Phe	340	5.3
32.0	Gly-Tyr-Ile	352	9.7
32.9	Tyr-Glu-Phe	458	10.2
33.8	Ala-Asp-Tyr	368	3.8
36.2	Glu-Gly-Gln	333	6.9
37.0	Gln-Phe-Ala	365	7.4
40.7	Phe-Met-Gly	354	5.2
40.8	Gly-Phe-Gly	280	7.5
42.9	Ile-Gly-Ser	276	5.5
45.8	Trp-Trp-Leu	504	5.1
46.2	Ala-Ala-Leu	274	9.3
46.9	Leu-Ileu-Phe	392	7.7
47.1	Ala-Leu-Ala	274	7.1
47.2	Pro-Gly-Thr	274	1.2
50.4	Phe-Leu-Met	410	5.7
52.6	Trp-Ala-Pro	373	7.1
62.4	Tyr-Ile-Ala	368	2.7
68.6	Phe-Ser-Pro	350	10.1
69.8	Phe-Phe-Tyr	476	1.3
75.6	Phe-Val-Ala	336	0.8
75.9	Gly-Phe-Ile	336	7.3
90.2	Ala-Ala-Val	260	2.5

大豆由来トリペプチドのＨＰＬＣにおける溶出時間、アミノ酸配列、分子量および阻害活性。　　＊【表３】

＊【００２１】

(単位 mmHg)

群	投与前血圧値	投与後時間（hr）				
		0.5	1	2	4	6
1群（蒸留水）	181.8	185.5	185.1	183.7	184.0	186.7
2群（300mg/kg	188.2	178.1	172.2	173.1	176.4	188.1
3群（600mg/kg	182.4	185.9	186.2	184.2	173.3	182.9
4群（1200mg/kg	186.4	185.4	182.8	181.8	170.9	179.8

有意差検定；＊危険率 5%，＊＊危険率 1%，＊＊＊危険率 0.1 %

イワシ筋肉由来トリペプチド分画投与におけるSHRの血圧値の経時的変化

＊【0022】
＊　【表4】

(単位 mmHg)

群	投与前血圧値	投与後時間（hr）				
		0.5	1	2	4	6
1群（蒸留水）	183.3	185.2	183.6	184.1	184.7	183.7
2群（300mg/kg	182.2	178.1	180.1	181.9	181.6	184.8
3群（600mg/kg	182.5	185.4	175.8	181.8	179.3	180.8
4群（1200mg/kg	181.8	187.4	175.0	179.2	179.8	181.6

有意差検定；＊危険率 5%，＊＊危険率 1%，＊＊＊危険率 0.1 %

大豆由来トリペプチド分画投与におけるSHRの血圧値の経時的変化

【0023】

【図面の簡単な説明】

【図1】本発明に係るイワシ筋肉由来トリペプチドの、製造例1におけるSP－Sephad－ex C－25（H^+）カラムクロマトグラフィーによるACE阻害ペプチドの分離精製の結果を示す図である。

【図2】本発明に係る大豆由来トリペプチドの、製造例1におけるSP－Sephadex C－25（H^+）カラムクロマトグラフィーによるACE阻害ペプチドの分離精製の結果を示す図である。

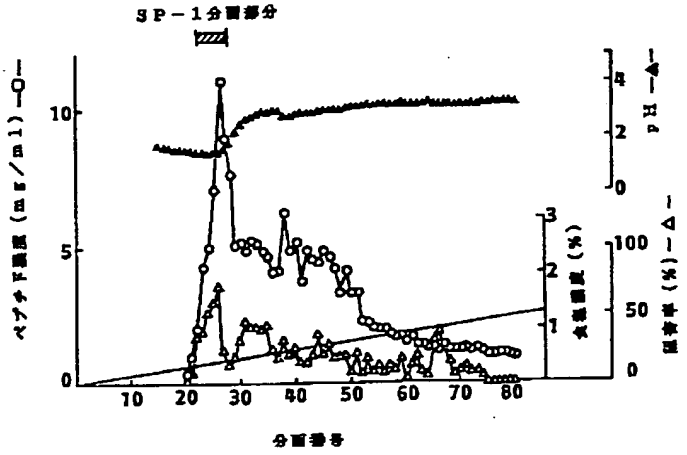
【図3】本発明に係るイワシ筋肉由来トリペプチドの、※製造例1における逆相HPLCによるACE阻害ペプチドの分離精製の結果を示す図である。

【図4】本発明に係る大豆由来トリペプチドの、製造例1における逆相HPLCによるACE阻害ペプチドの分離精製の結果を示す図である。

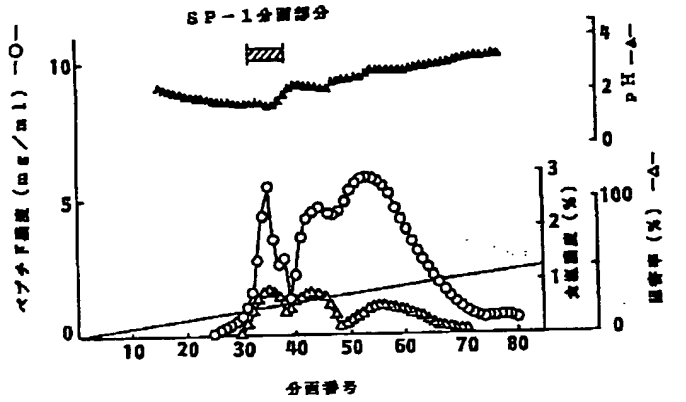
【図5】本発明に係るイワシ筋肉由来トリペプチドの製造例1で得られた23種のトリペプチド分画（SP－1分画）を、SHRに投与した場合の血圧値の経時的変化を示す図である。

【図6】本発明に係る大豆由来トリペプチドの製造例2で得られた27種のトリペプチド分画（SP－1分画）を、SHRに投与した場合の血圧値の経時的変化を示す図である。

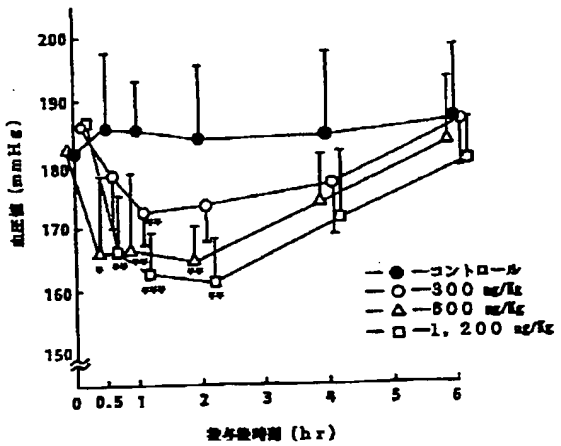
【図1】


SP－1分画部分
ペプチド濃度 (mg/ml) —○—
pH —▲—
食塩濃度 (%)
脱着率 (%) —△—
分画番号

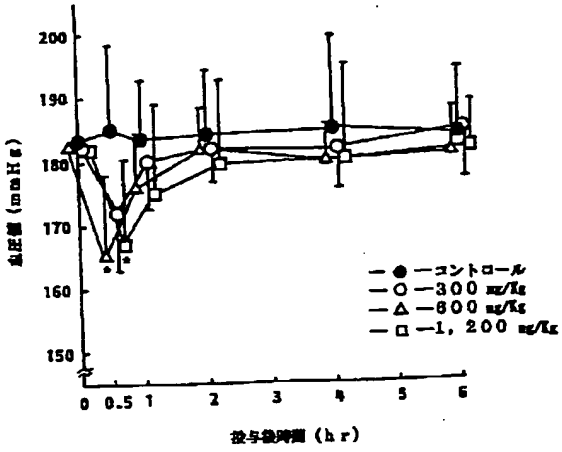
【図2】


SP－1分画部分
ペプチド濃度 (mg/ml) —○—
pH —▲—
食塩濃度 (%)
脱着率 (%) —△—
分画番号

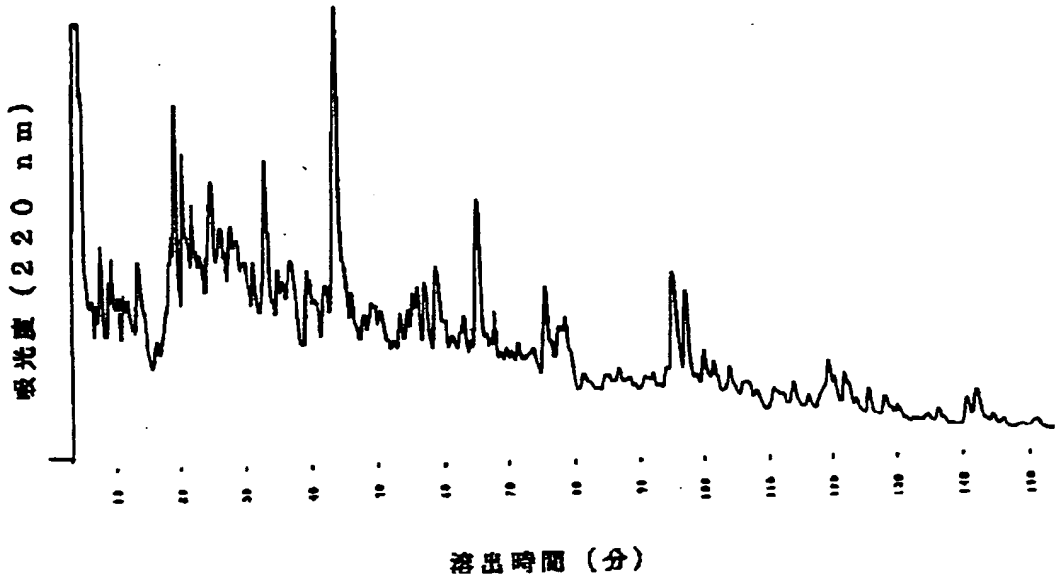
【図5】


血圧値 (mmHg)
—●—コントロール
—○—300 mg/kg
—△—600 mg/kg
—□—1, 200 mg/kg
投与後時間 (hr)

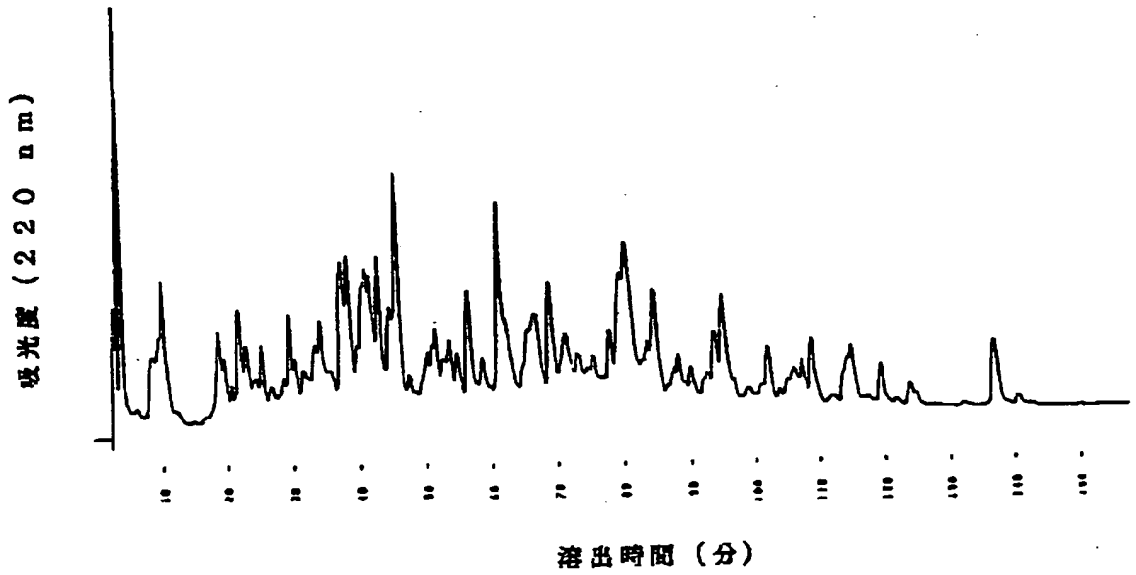
【図6】


血圧値 (mmHg)
—●—コントロール
—○—300 mg/kg
—△—600 mg/kg
—□—1, 200 mg/kg
投与後時間 (hr)

【図3】



【図4】



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(54) NOVEL TRIPEPTIDE, ITS PRODUCTION AND HYPOTENSOR CONTAINING THE SAME AS AN ACTIVE INGREDIENT

(57)Abstract:

PURPOSE: To obtain a novel tripeptide which is useful as a hypotensor with high safety, low toxicity and no anaphylaxy shock.

CONSTITUTION: Twenty three kinds of tripeptide having the L-amino acid sequences are represented by the formulas. The tripeptides are obtained by treating sardin muscles with a protease, filtering the product, and fractionating the components passing through the semipermeable membrane by means of a strong acid cation exchange resin, gel filtration, ion-exchange gel filtration, reverse-phase high-performance liquid chromatography in order to collect the fractions containing the components having the activity of inhibiting the enzymes transforming angiotensin.

Leu-Ala-Phe, Val-Ala-Tyr, Met-Val-Val,
Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,
Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

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[Date of extinction of right]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1]
次式; Leu-Ala-Phe, Val-Ala-Tyr, Met-Val-Val,
Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,
Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

23 kinds of new tripeptide which comes out and has the peptide structure by the array of the amino acid of L bodies shown.
[Claim 2] The process of 23 sorts of new tripeptide of claim 1 which filters the product which processed sardine muscles with protease and was obtained, carries out fractionation of the component which passed the semipermeable membrane in the **** component one by one with strongly acidic cation exchange resin, gel filtration, ion-exchange nature gel filtration, and reversed phase high pressure liquid chromatography, and is characterized by obtaining the fractionation containing the component which has angiotensin conversion enzyme inhibition activity from the fractionation obtained for the processing of every.
[Claim 3] The antihypertensive which makes an active principle one or more sorts of tripeptide chosen from 23 sorts of new tripeptide of claim 1.
[Claim 4]
次式; Ala-Ile-Met, Tyr-Ala-Val, Gly-Gly-Phe,
Gln-Gly-Phe, Leu-Glu-Leu, Tyr-Ala-Phe,
Gly-Tyr-Ile, Tyr-Glu-Phe, Ala-Asp-Tyr,
Glu-Gly-Gln, Gln-Phe-Ala, Phe-Met-Gly,
Gly-Phe-Gly, Ile-Gly-Ser, Trp-Trp-Leu,
Ala-Ala-Leu, Leu-Ileu-Phe, Ala-Leu-Ala,
Pro-Gly-Thr, Phe-Leu-Met, Trp-Ala-Pro,
Tyr-Ile-Ala, Phe-Ser-Pro, Phe-Phe-Tyr,
Phe-Val-Ala, Gly-Phe-Ile, Ala-Ala-Val,

27 sorts of new tripeptide which comes out and has the peptide structure by the array of the amino acid of L bodies shown.
[Claim 5] the component which filtered the product which processed the soybean with protease and was obtained, and passed the semipermeable membrane in the filtration component -- one by one -- strongly acidic

cation exchange resin, gel ****, ion-exchange nature gel filtration, and reversed phase high pressure liquid chromatography -- the process of 27 sorts of new tripeptide of claim 1 which carries out fractionation and is characterized by obtaining the fractionation containing the component which has angiotensin conversion enzyme inhibition activity from the fractionation obtained for the processing of every.
[Claim 6] The antihypertensive which makes an active principle one or more sorts of tripeptide chosen from 27 sorts of new tripeptide of claim 4.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the process of the antihypertensive which makes new tripeptide an active principle, and its new tripeptide.
[0002]
[Description of the Prior Art] Hypertension is divided roughly into what has the clear cause of elevation of blood pressure (****** hypertension), and an unknown thing (essential hypertension) in cause of a disease. Although hypertension can be made to recover because ****** hypertension makes the disease used as a cause recover, in essential hypertension, the direct cure to a cause is difficult. Conventionally, it is thought that a renin-angiotensin series (it is hereafter written as a R-A system.) is one of the important factors of essential hypertension, and the attempt which adjusts an R.A system and adjusts essential hypertension has been performed by checking the activity of the angiotensin converting enzyme (it is hereafter written as ACE.) which has played the central role by the R-A system since here ten years. As matter which has such ACE activity inhibition The compound which made the base A.Ondetti, B.Rubin et al;Science, 196,441(1977)], and it is known. the case of a synthetic compound -- L-proline derivative [-- M. -- Bradykinin enhancement factor (C terminal is Pro) [S. of the snake venom origin in the case of the matter of the natural product origin H.Ferreia, D.C.Bartelt et al;Biochemistry, 9, 3583(1970)], Six kinds of peptide (C terminal is Ala-Hyp) [G. of the collagenase digest origin of gelatin Oshima, H.Shimabukuro et al;Biochim.BioPhs, Acta, 566,128(1979)], Peptide (C terminal is Gly-Lys) [S. of the trypsin digest origin of cow casein Maruyama, H.Suzuki;Agric.Biol.Chem, 46, 1393(1982)], etc. are known. In the case of food, Suzuki and others accepts ACE activity inhibition from an soybean, tea, shellfish, and fruits (the formation of Takeo Suzuki and Nobuko Ishikawa; **, and [57, 1143] (1983)). However, although each matter of these natural product origin is [that effectiveness is only checked by intravenous administration, and], the pharmacology effectiveness by internal use is unknown, and a long period of time has passed after being invented, there is no report that the development as drugs is still progressing.
[0003]
[Problem(s) to be Solved by the Invention] The purpose of this invention is offering the antihypertensive which makes an active principle new tripeptide, its process, and it.
[0004]
[Means for Solving the Problem] In order that this invention may solve the aforementioned technical problem, as a result of inquiring wholeheartedly, the new peptide of this invention obtained from sardine muscles and the decomposition liquid of the protease of an soybean came to complete a header and this invention for having a blood-pressure descent operation. Namely, this invention,

(1)次式; Leu-Ala-Phe, Val-Ala-Tyr, Met-Val-Val,
Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,
Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

23 sorts of new tripeptide which comes out and has the amino acid sequence of L bodies shown.
[0005] (2) The process of 23 sorts of aforementioned new tripeptide which **** the product which processed sardine muscles with protease and was obtained, carries out fractionation of the component which passed the semipermeable membrane in the filtration component one by one with strongly acidic cation exchange resin, gel filtration, ion-exchange nature gel filtration, and reversed phase high pressure liquid chromatography, and is characterized by obtaining the fractionation containing the component which has angiotensin conversion enzyme inhibition activity from the fractionation obtained for the processing of every.
(3) The antihypertensive which makes the aforementioned new tripeptide an active principle.
(4)次式; Ala-Ile-Met, Tyr-Ala-Val, Gly-Gly-Phe,
Gln-Gly-Phe, Leu-Glu-Leu, Tyr-Ala-Phe,
Gly-Tyr-Ile, Tyr-Glu-Phe, Ala-Asp-Tyr,
Glu-Gly-Gln, Gln-Phe-Ala, Phe-Met-Gly,
Gly-Phe-Gly, Ile-Gly-Ser, Trp-Trp-Leu,
Ala-Ala-Leu, Leu-Ileu-Phe, Ala-Leu-Ala,
Pro-Gly-Thr, Phe-Leu-Met, Trp-Ala-Pro,
Tyr-Ile-Ala, Phe-Ser-Pro, Phe-Phe-Tyr,
Phe-Val-Ala, Gly-Phe-Ile, Ala-Ala-Val,

27 sorts of new tripeptide which comes out and has the amino acid sequence of L bodies shown.
[0006] (5) The process of 27 sorts of aforementioned new tripeptide which **** the product which processed the soybean with protease and was obtained, carries out fractionation of the component which passed the semipermeable membrane in the filtration component one by one with strongly acidic cation exchange resin, gel filtration, ion-exchange nature gel ****, and reversed phase high pressure liquid chromatography, and is characterized by obtaining the fractionation containing the component which has angiotensin conversion enzyme inhibition activity from the fractionation obtained for the processing of every.
(6) It is related with the antihypertensive which makes the aforementioned new tripeptide an active principle.
Hereafter, this invention is explained to a detail. New tripeptide of this invention,
次式; Leu-Ala-Phe, Val-Ala-Tyr, Met-Val-Val,
Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,
Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

(Each notation in 23 sorts and the formula of tripeptide shows each amino acid unit of the amino acid sequence in peptide chemistry above.)
[0007]

Ala-Ile-Met,	Tyr-Ala-Val,	Gly-Gly-Phe
Gln-Gly-Phe,	Leu-Glu-Leu,	Tyr-Ala-Phe
Gly-Tyr-Ile,	Tyr-Glu-Phe,	Ala-Asp-Tyr
Glu-Gly-Gln,	Gln-Phe-Ala,	Phe-Met-Gly
Gly-Phe-Gly,	Ile-Gly-Ser,	Trp-Trp-Leu
Ala-Ala-Leu,	Leu-Ileu-Phe,	Ala-Leu-Ala
Pro-Gly-Thr,	Phe-Leu-Met,	Trp-Ala-Pro
Tyr-Ile-Ala,	Phe-Ser-Pro,	Phe-Phe-Tyr
Phe-Val-Ala,	Gly-Phe-Ile,	Ala-Ala-Val

(-- each notation in 27 sorts and the formula of tripeptide shows each amino acid unit of the amino acid sequence in peptide chemistry above.) -- it is new tripeptide which has the amino acid sequence of L bodies shown, and the description in this ordinary temperature is white powder.
[0008] The approach of separating and refining from the decomposition liquid of the protease of an soybean as a process of the aforementioned new tripeptide in the approach or sardine muscular list which compounds the tripeptide chemically can be mentioned. Although it can carry out by the usual synthetic approaches, such as a liquid phase process or a solid phase technique, when compounding the new tripeptide of this invention chemically, it is good preferably to combine the amino acid of L bodies corresponding to the amino acid residue to the solid phase base material of polymer nature by peptide linkage one by one, and to go to it from the C terminal side (carboxyl-terminus side) of said tripeptide, by the solid phase technique. And after cutting the synthetic tripeptide obtained by making it such from the solid phase base material of polymer nature using trifluoro methansulfonic acid, hydrogen fluoride, etc., it can remove the protective group of an amino acid side chain, and can refine it by the usual approach using the high performance chromatography (it abbreviates to HPLC hereafter.) using the column of an opposition system etc.
[0009] Although separation purification of the new tripeptide of this invention can be carried out from the decomposition liquid of the protease of an soybean at a sardine muscular list, it is the collection P183 of the Japan Society for Bioscience, Biotechnology and Agrochemistry convention (Kyoto) lecture summaries in the 1991 fiscal year in that case. Based on the approach of 3AP13, it can carry out by [as being the following]. It hydrolyzes, after taking out an soybean in the sardine muscular partial list containing the above-mentioned new tripeptide and fully carrying out a homogenate using a homogenizer in suitable solvents (for example, neutral buffer solutions, such as water, the Tori Soe hydrochloric-acid buffer solution, and a phosphate buffer solution etc.). Hydrolysis is performed according to a conventional method. For example, after hydrolyzing further if it is in a sardine muscular homogenate list with the need about an soybean homogenate when hydrolyzing with protease, such as a pepsin, it warms to the optimum temperature of an enzyme and pH is adjusted to an optimum value, and an enzyme is added and it incubates. Subsequently, after neutralizing if needed, deactivation of the enzyme is carried out and hydrolysis liquid is obtained. An insoluble component is removed by filtering the hydrolyzate using a filter paper, cerite, etc. Semipermeable membrane, such as cellophane, is used for the obtained ****. A suitable solvent It fully dialyzes in inside. (For example, neutral buffer solutions, such as water and a tris-hydrochloric-acid buffer-solution phosphate buffer solution etc.) The solution containing the component which passed semipermeable membrane of the component in the **** is covered over strongly acidic cation exchange resin (for example, Dowex 50W by the Dow Chemical Co. etc.), and it is angiotensin converting enzyme (it abbreviates to ACE hereafter.) from the adsorption elution fractionation. The fractionation containing the component which has inhibition activity is obtained. The obtained ACE inhibition activity fractionation Gel **** Fractionation (is carried out [for example,] by Sephadex G-25 which are a Pharmacia manufacture). The obtained ACE inhibition activity fractionation Cation-exchange gel filtration It can carry out by (for example, carrying out by SP-Sephadex C-25 of a Pharmacia manufacture etc.)

fractionation, and carrying out fractionation of the obtained ACE inhibition activity fractionation by HPLC (reversed phase high pressure liquid chromatography) further.

[0010] Although a legume may be used for the fish muscular list used in the process of the new tripeptide of this invention at what kind of fish muscular list as long as the purpose of this invention can be attained as a legume, it is good to use an soybean for a sardine list preferably. Even if it prescribes a medicine for the patient repeatedly into a vein, an antibody production is not caused, and the new tripeptide of this invention obtained as mentioned above does not make an anaphylactic shock cause. Moreover, in order for the new tripeptide of this invention to consist of array structure of only L-amino acid, and to carry out the parenteral absorption for whether being Sumiya, without being decomposed by the protease in the living body after administration, in view of the molecule size and to demonstrate the blood-pressure descent operation, toxicity is very low, and safety is very high (fifty percent lethal dose) 5000 kg/kg; rat internal use. The new tripeptide concerning this invention can be adjusted to injections, a tablet, a capsule, a granule, powder, etc. using additives, such as an excipient usually used. Usually injecting the mammals (for example, Homo sapiens, a dog, a rat, etc.) which have ACE as a medication method, or administering orally are raised. A dose hits for example, the animal weight of 1kg, and is 0.01-10mg in amount about this tripeptide. Day, although the count of administration is about 1 - 4 times per, a route of administration can usually adjust it suitably. The new tripeptide concerning this invention has the outstanding angiotensin conversion enzyme inhibition operation, and shows a blood-pressure descent operation and bradykinin inactivation depressant action. Therefore, it is useful as prevention of hypertension, such as essential hypertension, renal hypertension, and adrenal hypertension, a therapy agent, and an antihypertensive used in the diagnostic agent and various kinds of symptoms of these diseases, and it has the normalization of organ circulation to congestive heart failure, and an improvement (prolongation-of-life effectiveness) operation of a long term prognosis further, and is useful as a therapy agent of cardiac insufficiency.

[Example] As an example, the example of manufacture and the example of a trial are indicated below, and this invention is further explained to it at a detail.

[0011] After adding and homogenizing deionized water 1L to 500g of example of manufacture 1 [manufacture from sardine muscles of new tripeptide] sardine muscles, 1-N hydrochloric acid adjusts pH to 2.0. Pepsin (Merck Co. make, enzyme number EC 3.4.23.1) 10g was added, and it hydrolyzed, agitating 37 degrees C for 20 hours. Ultrafiltration membrane (the Amicon make, YM10 mold, phi76mm) is made to pass decomposition reaction liquid immediately, and it is Dowex about passage liquid. It added to 50Wx4 [H+] column (phi4.5x15cm). After washing the column enough by deionized water, it was eluted using 2-N ammonium hydroxide liquid 2L. Vacuum concentration removed ammonia and 40ml of concentration liquid was obtained. Sephadex which buffer-ized 4ml of this concentration liquid by deionized water beforehand The load was carried out to G-25 column (phi2.5x150cm), and gel filtration was performed in rate-of-flow 30 ml/hr and each amount of fractionation of 8.6ml. Fractions with the high ACE inhibition activity which repeated and carried out extensive preparative isolation of the gel **** were collected, and it freeze-dried, and considered as peptide powder. SP-Sephadex which buffer-ized this peptide 3g by deionized water beforehand after dissolving in 20ml deionized water The load was carried out to C-25 (H+) column (phi1.5x47.2cm), the concentration gradient method of deionized water 1L to 3% sodium chloride liquid 1L was performed, and the chromatography was performed in rate-of-flow 3 ml/hr and each amount of fractionation of 10.0ml. The result is as being shown in drawing 1 . Among the above-mentioned chromatograph, the ACE inhibition activity fractionation of the fractionation numbers 22-28 was collected, it freeze-dried, and purification tripeptide powder was obtained. HPLC was performed after dissolving 20mg of this purification tripeptide powder in the deionized water of 60microl. as a column -- the product made from Nomura Chemistry -- DevelosilODS-5 (4.5mmIDx25cmL) was used, as a mobile phase, 25%, the concentration gradient method of TFA was performed 0.05%, the chromatography was performed on rate-of-flow 1.0 ml/min and the detection wavelength of 220nm, and an acetonitrile / tripeptide which has ACE inhibitory action was obtained from trifluoroacetic acid (it is hereafter written as TFA.) 0.05%. The result is as being shown in drawing 2 , and the elution time amount of 23 sorts of tripeptide is as in Table 1.

[0012] Thus, the amino acid sequence of the tripeptide which has the obtained ACE inhibitory action was determined using the Applied Biosystem amino acid sequence analyzer 447A mold. Consequently, 23 sorts of

次式; Leu-Ala-Phe, Val-Ala-Tyr, Met-Val-Val,
Val-Val-Leu, Ala-Ala-Phe, Leu-Ala-His,
Leu-Glu-Leu, Ala-Tyr-Val, Ala-Val-Met,
Ala-Val-Lys, Glu-Val-Tyr, Gly-Val-Leu,
Tyr-Asp-Ala, Leu-Trp-Trp, Leu-Ala-Ala,

tripeptide is each,

Glu-Ala-Val, Phe-Ile-Leu, Ala-Leu-Ala,
Thr-Gly-Pro, Met-Gly-Ile, Leu-Ala-Val,
Leu-Val-Val, Asn-Gln-Phe,

It was checked that it is tripeptide which has the array which comes out and consists of amino acid residue of L bodies shown. As a result of ****(ing) the tripeptide of new 23 kinds with a mass spectrum, it was checked that an amino acid sequence and amino acid composition are tripeptide which has the amino acid sequence structure shown by said formula. The result of this mass spectrum is as being shown in Table 1.

[0013] After adding and homogenizing deionized water 1L to 200g of the example 2 [manufacture from soybean of new tripeptide] soybeans of manufacture, 1-N hydrochloric acid adjusted pH to 2.0, and pepsin (Merck Co. make, enzyme number EC 3.4.23.1) 10g was added, and it hydrolyzed, agitating 37 degrees C for 20 hours. Extra ****** (the Amicon make, YM10 mold, phi76mm) is made to pass decomposition reaction liquid immediately, and it is Dowex about passage liquid. It added to 50Wx4 [H+] column (phi4.5x15cm). After washing the column enough by deionized water, it was eluted using 2-N ammonium hydroxide liquid 2L. Vacuum concentration removed ammonia and 40ml of concentration liquid was obtained. Sephadex which ****-ized 4ml of this concentration liquid by deionized water beforehand The load was carried out to G-25 (phi2.5x150cm), and gel filtration was performed in rate-of-flow 30 ml/hr and each amount of fractionation of 8.6ml. Fractionation with the high ACE inhibition activity which repeated and carried out extensive preparative isolation of the gel filtration was collected, and it freeze-dried, and considered as peptide powder. SP-Sephadex which buffer-ized this peptide 3g by deionized water beforehand after dissolving in 20ml deionized water The load was carried out to C-25 [H+] column (phi1.5x47.2cm), the concentration gradient method of deionized water 1L to 3% sodium chloride liquid 1L was performed, and the chromatography was performed by rate-of-flow 3 ml/hr and 10.0ml of each fractionation. The result is as being shown in drawing 3 .

[0014] Among the above-mentioned chromatograph, the ACE inhibition activity fractionation of the fractionation numbers 32-38 was collected, it freeze-dried, and purification tripeptide powder was obtained. HPLC was performed after dissolving 20mg of this purification tripeptide powder in the deionized water of 60microl. as a column -- the product made from Nomura Chemistry -- DevelosilODS-5 (4.5mmIDx25cmL) was used, as a mobile phase, 25%, the concentration gradient method of TFA was performed 0.05%, chromatography GURAI was performed on rate-of-flow 1.0 ml/min and the detection wavelength of 220nm, and an acetonitrile / RIPEPUCHIDO which has ACE inhibitory action was obtained from trifluoroacetic acid (it outlines Following TFA.) 0.05%. The result is as being shown in drawing 4 , and the elution time amount of 27 sorts of tripeptide is as in Table 2.

[0015] Thus, the amino acid sequence of the tripeptide which has the obtained ACE inhibitory action was determined using the Applied Biosystem amino acid sequence analyzer 477A mold. Consequently, 27 sorts of

次式； Ala-Ile-Met, Tyr-Ala-Val, Gly-Gly-Phe,
Gln-Gly-Phe, Leu-Glu-Leu, Tyr-Ala-Phe,
Gly-Tyr-Ile, Tyr-Glu-Phe, Ala-Asp-Tyr,
Glu-Gly-Gln, Gln-Phe-Ala, Phe-Met-Gly,
Gly-Phe-Gly, Ile-Gly-Ser, Trp-Trp-Leu,
Ala-Ala-Leu, Leu-Ileu-Phe, Ala-Leu-Ala,
Pro-Gly-Thr, Phe-Leu-Met, Trp-Ala-Pro,
Tyr-Ile-Ala, Phe-Ser-Pro, Phe-Phe-Tyr,
tripeptide is each, Phe-Val-Ala, Gly-Phe-Ile, Ala-Ala-Val,

It was checked that it is tripeptide which has the array which comes out and consists of amino acid residue of L bodies shown. As a result of ****(ing) the tripeptide of new 27 kinds with a mass spectrum, it was checked that an amino acid sequence and amino acid composition are tripeptide which has the amino acid sequence structure shown by said formula. The result of this mass spectrum is as being shown in Table 2. The pharmacology effectiveness was checked by the trial which shows below the fractionation which consists of 23 sorts of sardine muscular origin tripeptide concerning this invention refined and obtained, and the fractionation which changes from 27 sorts of soybean origin tripeptide to a list.

[0016] It measured according to the approach (a Japanese **** meeting magazine, 18,297-302 (1989)) of Yamamoto which improved the measuring method of Lieberman using example of trial 1 [ACE inhibition activity measurement method] ACE(sigma company make, enzyme number EC 3.4.15.1)2.5mU, and synthetic substrate Hippuryl-L-his-tidyl-L-leucine(made in peptide lab) 12.5mM. That is, ethyl acetate extracted the generated hippuric acid and it measured with the absorbance of 225nm. It asked for the rate of inhibition from the degree type, having used Ec and the value at the time of adding the reaction stop solution and making it react beforehand as Eb for the value when applying the absorbance in sample liquid for the buffer solution instead of Es and sample liquid.

The concentration (M) of a sample required in order to carry out inhibition of the enzyme activity of ACE 50% (rate of inhibition) showed inhibition activity IC50 value of a rate (%) of inhibition =(Ec-Es)/(Ec-Eb)x100ACE inhibitor. Inhibition activity over the bovine lung blood serum ACE of the tripeptide of sardine muscular origin new 23 kinds concerning this invention (IC50) It is as being shown in Table 1. Moreover, the inhibition activity (IC50) over the bovine lung blood serum ACE of the tripeptide of soybean origin new 27 kinds concerning this invention is as being shown in Table 2.

[0017] The example 2 of a trial [effectiveness of pressure lowering at the time of administration to the rat of new tripeptide]

I. Purification tripeptide powder obtained in the examples 1 and 2 of the subjects-of-an-experiment aforementioned manufacture. That is, the fractionation (SP-1 fractionation) which changes from 27 sorts of soybean origin tripeptide to the fractionation (SP-1 fractionation) list which consists of 23 sorts of sardine muscular origin tripeptide was used.

From Charles River Japan, INC., the II. experiment approach laboratory animal purchased 15 weeks-old male hypertension natural onset rat (SHR), and systolic blood pressure used it after preliminary breeding for one week as six animal one group of 160 or more (weights 280-330g) mmHgs. It held one rat at a time in the individual cage for the rats made from a stainless steel wire, and it was bred in it at the breeding room adjusted to the room temperature of 23**25 degrees C, 55**10% of humidity, and 12-hour light and darkness (6:00 a.m. - 6:00 lighting). Drinking water made private pumping (drinking water standard adaptation) take in [feed] Oriental Yeast Works MF powder feed freely, respectively. Divided the rat into four groups (one groups [six]), and carried out forcible internal use of distilled water at a rate of 0.5ml per weight of 100g as contrast at the 1st group. In the 2nd group, the dosage of powder 1.0 g/kg of tripeptide is prepared with distilled water. Forcible internal use was carried out at a rate of 0.5ml per weight of 100g, powder 2.0 g/kg of tripeptide was carried out at the 3rd group, and forcible internal use of the dosage of powder 4.0 g/kg of tripeptide was carried out like the 2nd group at the 4th group.

[0018] blood pressure -- bloodlessness caudal artery blood-pressure-measurement equipment (Product made

from the Riken Development, PS-100) -- using -- tail-cuff -- by law, the blood pressure and the heart rate of after [administration] 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours were measured before administration. Blood pressure was measured three continuation, and when the difference of the peak price and minimum value was less than 10 mmHgs, 3 times of the mean-blood-pressure value was calculated. When a difference was 11 or more mmHgs, it measured twice [further], and except for a peak price and the minimum value, 3 times of mean-blood-pressure values were calculated. Moreover, it asked for the average heart rate using the measured value when computing a mean-blood-pressure value. The result about the operation to the blood-pressure value and heart rate when carrying out single time internal use of the sardine muscular origin tripeptide fractionation (SP-1 fractionation) 300,600 and 1,200 mg/kg using SHR is as being shown in Table 3 and drawing 5 . Moreover, it is as the result about the operation to the blood-pressure value and heart rate when carrying out single time internal use of the soybean origin tripeptide fractionation (SP-1 fractionation) 300,600 and 1,200 mg/kg being shown in Table 4 and drawing 6 using SHR. As a result of the above trial, the fractionation which changes from 27 sorts of soybean origin tripeptide to the fractionation list which consists of 23 sorts of sardine muscular origin tripeptide concerning this invention has ACE inhibition activity, and is in. It was checked that a significant blood-pressure descent operation is shown also in vivo. Therefore, 27 sorts of soybean origin tripeptide is useful as the therapy or prophylactic of hypertension in 23 sorts of sardine muscular origin tripeptide list concerning this invention. In addition, 27 sorts of soybean origin tripeptide can also adopt the amino acid sequence as 23 sorts of sardine muscular origin tripeptide list concerning this invention into structure in the peptide made into a substructure structurally.

[0019]

[Table 1]

HPLCにおける溶出時間（分）	アミノ酸配列	分子量（MH+）	阻害活性 IC_{50}値（$\times 10^{-5}$M）
20.0	Leu-Ala-Phe	350	5.3
21.8	Val-Ala-Tyr	352	4.2
30.7	Met-Val-Val	348	3.1
30.9	Val-Val-Leu	330	2.8
31.3	Ala-Ala-Phe	308	9.2
31.6	Leu-Ala-His	340	8.3
31.8	Leu-Glu-Leu	374	1.3
31.9	Ala-Tyr-Val	352	1.7
32.1	Ala-Val-Met	320	0.8
32.6	Ala-Val-Lys	317	1.6
32.7	Glu-Val-Tyr	410	8.1
32.8	Gly-Val-Leu	288	8.8
33.8	Tyr-Asp-Ala	308	4.7
44.8	Leu-Trp-Trp	504	5.6
44.9	Leu-Ala-Ala	274	6.1
55.2	Glu-Ala-Val	318	6.8
55.9	Phe-Ile-Leu	302	1.9
60.0	Ala-Leu-Ala	274	3.8
60.1	Thr-Gly-Pro	274	5.3
60.2	Met-Gly-Ile	320	5.6
72.0	Leu-Ala-Val	302	4.8
72.5	Leu-Val-Val	330	6.5
88.4	Asn-Glu-Phe	408	4.9

The elution time amount in HPLC, the amino acid sequence, molecular weight, and inhibition activity of sardine muscular origin tripeptide.

[0020]

[Table 2]

HPLCにおける溶出時間（分）	アミノ酸配列	分子量（MH⁺）	阻害活性 IC_{50}値（$\times 10^{-5}$M）
19.1	Ala-Ile-Met	334	0.3
21.8	Tyr-Ala-Val	352	4.6
28.6	Gly-Gly-Phe	280	2.1
31.6	Gln-Gly-Phe	351	6.7
31.8	Leu-Glu-Leu	374	8.1
31.9	Tyr-Ala-Phe	340	5.3
32.0	Gly-Tyr-Ile	352	9.7
32.9	Tyr-Glu-Phe	458	10.2
33.8	Ala-Asp-Tyr	368	3.8
36.2	Glu-Gly-Gln	333	6.9
37.0	Gln-Phe-Ala	365	7.4
40.7	Phe-Met-Gly	354	5.2
40.8	Gly-Phe-Gly	280	7.5
42.9	Ile-Gly-Ser	276	5.5
45.8	Trp-Trp-Leu	504	5.1
46.2	Ala-Ala-Leu	274	9.3
46.9	Leu-Ileu-Phe	392	7.7
47.1	Ala-Leu-Ala	274	7.1
47.2	Pro-Gly-Thr	274	1.2
50.4	Phe-Leu-Met	410	5.7
52.6	Trp-Ala-Pro	373	7.1
62.4	Tyr-Ile-Ala	368	2.7
68.6	Phe-Ser-Pro	350	10.1
69.8	Phe-Phe-Tyr	476	1.3
75.6	Phe-Val-Ala	336	0.6
75.9	Gly-Phe-Ile	336	7.3
90.2	Ala-Ala-Val	280	2.5

The elution time amount in HPLC, the amino acid sequence, molecular weight, and inhibition activity of soybean origin tripeptide.

[0021]

[Table 3]

(単位 mmHg)

群	投与前血圧値	投与後時間 (hr)				
		0.5	1	2	4	6
1群 (蒸留水)	181.8	185.5	185.1	183.7	184.0	188.7
2群 (300mg/kg	186.2	178.1	172.2	173.1	176.4	186.1
3群 (600mg/kg	182.4	185.9	186.2	184.2	173.3	182.9
4群 (1200mg/kg	186.4	185.4	182.8	181.8	170.9	179.8

有意差検定; *危険率 5%, **危険率 1%, ***危険率 0.1 %

Change of the blood-pressure value of SHR in sardine muscular origin tripeptide fractionation administration with time [0022]

[Table 4]

(単位 mmHg)

群	投与前血圧値	投与後時間 (hr)				
		0.5	1	2	4	6
1群 (蒸留水)	183.3	185.2	183.8	184.1	184.7	183.7
2群 (300mg/kg	182.2	178.1	180.1	181.9	181.6	184.8
3群 (600mg/kg	182.5	185.4	175.8	181.8	179.3	180.8
4群 (1200mg/kg	181.8	187.4	175.0	179.2	179.8	181.8

有意差検定; *危険率 5%, **危険率 1%, ***危険率 0.1 %

Change of the blood-pressure value of SHR in soybean origin tripeptide fractionation **** with time [0023]

[Translation done.]